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OBSERVATIONS OF MOUSE FETUSES AFTER IRRADIATION WITH 2.45 GHz MICROWAVES

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Abstract—Pregnant CD-1 mice were exposed to 2.45 GHz CW radiation for 100 min daily at a range of power densities (3.4–28 mW/cm^2). Near-term fetuses were examined for gross external morphologic alterations. Mean live fetal weight per litter decreased significantly with exposure to the highest power density (sham, 0.97 ± 0.15 g; irradiated, 0.89 ± 0.13 g). There was a significantly increased incidence of cranioschisis in exposed fetuses. An exposure of the dam for 100 min at these power densities did not appear to be significant thermally. Estimates of mean dose rate as determined using twin-well calorimetry ranged from 2.0 to 22.2 mW/g.

INTRODUCTION

THE PUBLIC health interest of the Environmental Protection Agency in electromagnetic radiation extends to the possible effects in the fetus at levels of exposure reasonably expected in the environment. This study is an attempt to fulfill that interest with a series of experiments using a range of power densities approaching present occupational protection guidelines.

The report by Pobzhitkov *et al.* (Po61) contained data of the deleterious morphologic effects in mice irradiated *in utero* with a super high frequency (SHF) microwave field. Since then, there has emerged a significant number of both positive and negative reports of the teratologic nature of microwave radiations. The intense investigative interest was demonstrated by the publication of nine studies' results in 1975–1976, almost as many as in the previous 15 yr. With this report, we hope to contribute to the understanding of microwave bio-effects.

MATERIALS AND METHODS

Virgin male and female adult CD-1* mice were caged together for breeding purposes and maintained on Purina Laboratory Chow† *ad lib.* Water was furnished through an automatic watering system; deionized water was supplied to the animals in the 3.4 and 13.6 mW/cm^2 and ordinary tap water to those in the 14 and 28 mW/cm^2 power density experiments. Daily between 0800 and 0900 hr, the females were examined for copulatory plugs and, if present, were assigned alternately to sham-irradiated or irradiated experimental groups, identified, weighed, and housed by sham-irradiated/irradiated experimental pairs. The day on which the copulatory plug was found was counted as day 1 after breeding. No male contributed more than 3 breedings. Bred females weighed between 25 and 33 g.

Subjects were treated to daily irradiation for 100 min between 0930 and 1530 hr in an anechoic 2.45 GHz CW microwave exposure facility described by others (El75; Bl75). A second controlled environment chamber was used to treat the sham subjects and was adjacent to the anechoic chamber; the

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controlled air was circulated through both environmental chambers so that the ambient air conditions were common. The temperature and humidity were maintained at 20.2 ± 0.5°C and 50% humidity. The incident power density was determined with the use of an *E*-field dipole probe (E175) placed at the center of the field in the plane at which the animals were exposed. Table 1 contains a summary of the conditions at each of the 4 power densities used in this study. All the mice were killed on day 18 after breeding. At that time, pregnancy was ascertained, and the uterus examined for the number of resorbed and dead conceptuses and live fetuses. The live fetuses were removed, examined for gross external morphologic alterations, blotted dry and weighed. During the daily 100-min period of treatment individual mice were caged in small containers. Each cage was 6 cm on a side, 1.5 mm thick, clear polycarbonate plastic, with a 1-mm-thick PVC plastic lid. Each side was drilled with 6–8 ventilation holes, 5 mm in diameter. The plastic cages were placed in a styrofoam form to ensure their exact positioning in the field (Fig. 1). The dimensions of the exposure configurations, called arrays, are in Table 2. The exposures of animals were carried out between September 1974 and December 1976.

The specific absorption rate (SAR) was determined using the twin-well calorimetric technique described by Kinn (Ki77). Briefly, the technique requires the use of two animal carcasses of the same mass, one of which has been exposed to microwave radiation while aligned parallel to the *E*-field and thus heated somewhat. The difference in caloric content between the unexposed and exposed carcasses is determined with a twin-well calorimeter and this value converted to the appropriate SAR units. The SAR value for each array position was determined with the remaining cells occupied with live animals. Statistical analysis of values for positions and arrays was done using analysis of variance techniques and regression analysis.

Statistical analyses on enumerative reproduction data were done using contingency tables. Continous fetal variables were analyzed with analysis of variance and covariance techniques. Statistical comparisons were made only between irradiated and sham groups at each exposure level. No attempt was made to pool data across exposure level groups, except in the case of the total incidence of any anomalous or craniosclistic fetuses. Values are reported by litter.

The rectal temperatures of naive CD-1 female mice were measured just before and just after sham or irradiation conditions with power densities of 3.4, 13.6, or 28 mW/cm^2 for 100 min employing the relevant exposure array. Temperatures were determined with a YSI Model 402 thermistor probe and a Model 46 telethermometer. Statistical comparisons at each power density were made using analysis of variance techniques.

RESULTS

The pregnancy rates for sham and irradiated bred dams were similar for each exposure group (Table 3). There was a difference between the pregnancy rates of the 3.4 mW/cm^2 groups and other power densities due to the inadvertent use of young males for this experiment. The means of live, dead, or total fetuses per litter, or ratio of litters with or without dead fetuses, could not be shown to be significantly different when sham and

Table 1. Summary of experimental conditions

POWER DENSITY (mW/cm^2)	GESTATIONAL DAYS OF EXPOSURE	EXPOSURE ARRAY (columns X rows)	ANIMAL POSITION IN ARRAY	NUMBER OF LITTERS	
				SHAM	TREATED
3.4	1-17	5 x 5	Same Daily	117	103
13.6	1-17	5 x 5	Same Daily	106	109
14	1-17	7 x 4	Random Daily	73	62
28	6-15	3 x 5	Same Daily	40	44
			(TOTALS)	336	318

CORRIGENDUM

Health Physics 35(6): 791-801, 1978.

1. page 797, next to last line: "38 mW/cm^2" should read "38 mW/g".
2. page 798, line 15: "30 mW/cm^2" should read "30 mW/g".
3. page 800, in Summary: "1.2-13.3 J/g" should read "12-133 J/g".

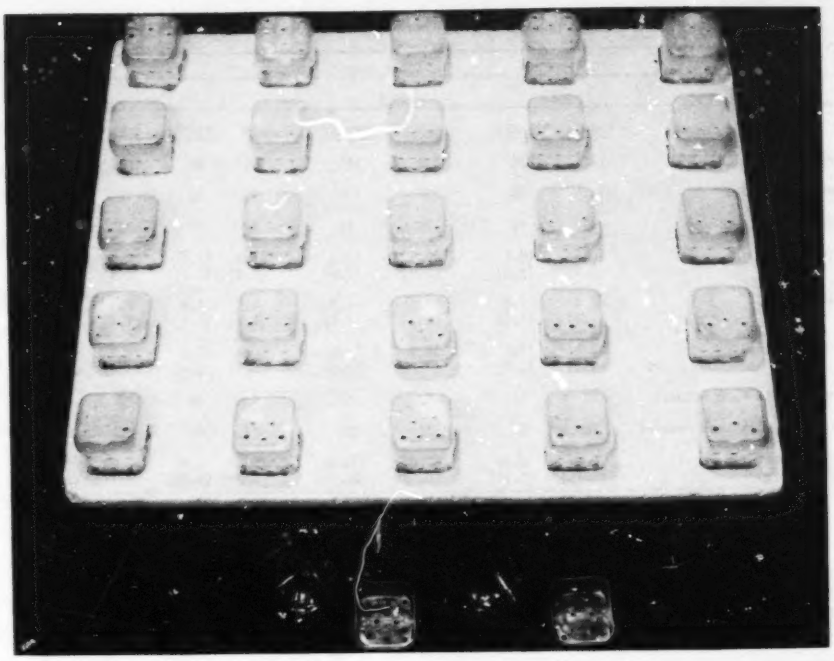

FIG. 1. Exposure array used at 3.4 and 13.6 mW/cm^2. Two boxes, with covers removed, are in the foreground.

Table 2. Dimensions of exposure arrays at each power density

POWER DENSITY (mW/cm^2)	EXPOSURE ARRAY COLUMNS	ROWS	OUTSIDE LIMITS (cm)	DISTANCE (cm) BETWEEN: COLUMNS	ROWS
3.4	5	5	69W x 56D	6	9.5
13.6	5	5	69W x 56D	6	9.5
14	7	4	60W x 32D	3	3
28	3	5	46W x 33D	13.5	1

W = Width; D = Depth.

irradiated groups were compared at each power density.

The litter mean live fetal weight was significantly ($P = 0.008$) decreased in the group irradiated with 28 mW/cm^2 when compared to its shams (Table 3). The mean value for these irradiated litters was also lower than the mean of any other sham or irradiated group studied. Sham-to-irradiate comparisons of the mean live fetal weight within each power density were conducted after correction for litter size. Others (Gr66) have reported a fetal weight:litter size relationship, and correction of mean fetal weight is a convention in sufficiently large populations. The factors for this correction technique (Table 4) were found by analysis of covariance using regression analysis methods. Litters in the two lower power densities displayed significant regression of live fetal weight values associated with litter size. Values for litters in the two higher power densities were corrected for litter size as well, even though no significant differences were found in their cases; this was accepted on the basis that the population size employed at the two higher power densities was too small to allow the statistical

OBSERVATIONS OF MOUSE FETUSES

Table 3. Results of examination of litters at various power densities

	Power Density (mW/cm^2)							
	3.4		13.6		14		28	
VARIABLE	SHAM	TREATED	SHAM	TREATED	SHAM	TREATED	SHAM	TREATED
Bred Females	278	225	144	143	106	89	61	62
Pregnant Females	117	103	106	109	73	62	40	44
% Pregnant	42	46	74	76	69	70	66	71
Live/Litter	10.1* (3.9)**	10.1 (3.5)	10.6 (3.0)	10.9 (2.9)	11.2 (2.4)	11.0 (2.1)	10.1 (2.9)	10.3 (2.7)
Dead/Litter	1.4 (1.4)	1.4 (1.3)	1.5 (1.3)	1.7 (1.4)	1.6 (1.4)	1.6 (1.4)	1.3 (1.4)	1.5 (1.5)
Total Implants/Litter	11.5 (4.3)	11.5 (3.6)	12.1 (3.2)	12.6 (2.6)	12.8 (2.5)	12.5 (2.4)	11.4 (3.1)	11.8 (2.8)
Litters with Dead Fetuses	82	77	82	87	53	46	27	34
% Litters with Dead Fetuses	70	75	77	80	73	74	68	77
Litter Mean Live Fetal Weight (g)	0.97 (0.10)	0.98 (0.15)	1.01 (0.13)	0.98 (0.12)	0.98 (0.28)	0.96 (0.14)	0.97 (0.15)	0.89 (0.13)

*Mean.
**Standard deviation.

Table 4. Estimated decrease in mean fetal weight for one fetus increase in litter size

POWER DENSITY (mW/cm^2)	DECREASE (Grams/Litter)
3.4	0.0084 ($p<0.001$)
13.6	0.0102 ($p<0.001$)
14	0.0059
28	0.0011

Table 5. Litter occurrence of external morphologic defects in living mouse fetuses at various power densities. Multiple defects are listed only once

	POWER DENSITY (mW/cm^2)									
VARIABLE	3.4		13.6		14		28		TOTAL LITTERS	
	SHAM	TREATED	SHAM	TREATED	SHAM	TREATED	SHAM	TREATED	SHAM	TREATED
Total Litters	117	103	106	109	73	62	40	44	336	318
Anomaly										
Hematoma	0	4	3	2	1	0	1	0	5	6
Omphalocele	0	0	0	1	0	0	0	0	0	1
Kinked Tail	1	0	0	1	0	1	0	0	1	2
Small Jaw	0	0	0	0	0	1	0	0	0	1
Club-foot	1	3	1	0	0	0	0	2	2	5
Open Eye(s)	0	1	2	1	0	0	0	0	2	2
Syndactyly	0	0	1	0	0	0	0	0	1	0
Palatoschisis	0	0	0	0	0	0	1	1	1	1
Schistocele	0	1	0	1	0	0	0	0	0	2
Cranioschisis	0	1	0	1	0	3	0	2	0	7
Affected Litters	2	10	7	7	1	5	2	5	12	27

significance to be demonstrated by these methods.

At the time of removal from the uterus, the living fetuses were examined externally for morphologic defects. The occurrence by litter of the various anomalous conditions observed are in Table 5. The conditions are arranged by increasing order in our own estimation of severity. Any litter which contained multiple defects was scored only once in the most severe category. Statistical significance ($P = 0.009$) was shown for the difference in occurrence of anomalous litters at 3.4 mW/cm^2. No significant difference in the distribution of anomalous litters could be shown at the other power densities.

The occurrence of cranioschisis was not different statistically at any one power density. However, when the occurrence of this anomaly was summed by all sham or irradiated groups, there was a significantly ($P = 0.007$) higher number in the irradiated group (sham = 0, irradiated = 7). The total numbers of sham or irradiated litters with any anomaly were significantly ($P = 0.008$) different. More than twice as many anomalous litters were seen in the irradiated group.

The mean rectal temperatures taken before and after sham or irradiation are in Table 6. The rectal temperatures of sham-exposed mice decreased approx 0.8°C; the arithmetic discrepancy in ΔT for this group is caused by rounding-off of values. Those mice exposed to 3.4 and 13.6 mW/cm^2 decreased similarly to shams. On the other hand, mice exposed to 28 mW/cm^2 for 100 min sustained their pre-exposure rectal temperature.

The mean SAR, as determined with twin-well calorimetry, is shown for the 5×5 and 3×5 arrays in Table 7. These values are related to a single-power density measurement made at the center of the array on axis with the horn antennae, without the presence of animals in the chamber. Although individual array positions were not considered in the effect relationships for this study, it is proper for the sake of completeness to include the variations of SAR as a function of position (Table 8). In this data the incident power density measurement was made for each position of the 5×5 array. Each dose rate value was therefore adjusted for the actual incident power level to that position when the center position received 10 mW/cm^2. The variation of dose rate is small enough at the 3.4 and 13.6 mW/cm^2 exposure levels to assure us of a significantly different absorption rate with no overlap in their distributions.

Table 7. Dose absorbed by 25–35 g mice in various exposure arrays

Array	Power Density (mW/cm^2)	SAR (mW/g) Mean	SD	No. of Mice
5 x 5	3.4	2.0	.5	171
5 x 5	13.6	8.1	2.2	171
3 x 5	28	22.2	5.6	47

DISCUSSION

The microwave literature pertinent to this study is limited here to that which employed rats or mice as the experimental subject. We would prefer to review these reports in a more pertinent order than chronologic, but this is not possible due to the variations of employed radio-frequency, power density, time of exposure, and subject species, among other experimental conditions.

In a study of mice exposed to a non-descript pulsed SHF field for 10 min daily during days 1–12 of pregnancy, at a power

Table 6. Rectal temperatures (°C) of adult CD-1 mice before and after 100 min of sham or irradiation with 2.45 GHz. Values are means and standard deviation

	POWER DENSITY (mW/cm^2)			
	SHAM	3.4	13.6	28
Pre-Treatment	38.1 ± .5	37.9 ± .4	38.7 ± .4	38.1 ± .3
Post-Treatment	37.4 ± .5	37.5 ± .4	37.8 ± .5	38.2 ± .6
ΔT	-.8 ± .6	-.4 ± .5	-.9 ± .7	+.1 ± .6
Number of Animals	87	18	18	21

Table 8. SAR variations in mice in the cells of the 5 × 5 array. Power density = 10 mW/cm² [a = mean sar (mW/g); b = number of values; c = standard deviation; d = position]

1[d] 7.16[a] 1.63[c] (8)[b]	2 5.04 1.54 (7)	3 4.36 0.79 (6)	4 6.62 1.37 (7)	5 6.77 1.34 (11)
6 6.03 0.32 (6)	7 6.48 1.09 (6)	8 4.05 0.91 (7)	9 5.45 1.22 (6)	10 5.95 1.05 (8)
11 7.37 1.25 (7)	12 5.81 1.49 (8)	13 5.99 1.87 (9)	14 6.66 0.88 (6)	15 6.31 1.15 (6)
16 4.86 0.93 (6)	17 4.72 0.83 (6)	18 5.77 1.83 (8)	19 6.01 1.15 (6)	20 5.89 1.85 (7)
21 5.69 1.60 (6)	22 5.18 1.42 (6)	23 6.10 1.08 (6)	24 7.37 2.99 (6)	25 5.11 2.03 (6)

density of 50 mW/cm², Pobzhitkov *et al.* (Po61) reported a number of changes in the pregnancies and the offspring. Among the changes in the 35 irradiated dams were a tendency towards lengthened gestation (1–3 days longer than controls) and delayed growth at weaning (mean body weight of control offspring, 9.2 g; irradiated offspring, 6 g). The importance of this paper, besides the demonstration of deleterious reproductive results, is in the larger size (35 treated pregnancies) of the experimental sample. The report, unfortunately, is incomplete in its lack of statistical data and stated radio-frequency conditions employed (the Russian use of "SHF" as a specific radio-frequency has been defined as 300–300,000 MHz) (Ba76, p. 13). It is interesting to note as well the short gestation period (18 days) of the mice used as controls, as compared to mice used in the U.S. Another Soviet report (Go64) noted lowered fertility, smaller litters, increased incidence of anomalous offspring and stillbirths, and decreased pregnancy rate among female mice irradiated with a pulsed (577 cycles/sec) 3-cm wave at 400 mW/cm² for 5 min at unspecified times. Controls were heated in an incubator at 52°C to affect similar rectal and skin temperatures as the irradiated mice. This heating caused reproductive impairment, but not as severe as that in irradiates.

The subthermal studies by Bereznitskaya (Be68, Be73) of 21 mice exposed 2 hr daily on days 1–15 of pregnancy (10 cm wavelength, 10 mW/cm²) resulted in a severe increase in fetal wastage. The mean pre- and post-implantation loss in the irradiated dams exceeded 28% of the ova shed, while the total loss in the 21 controls was only 11.5%. The lowered mean number of live fetuses reflected this excessive reproductive wastage (control, 7.8; irradiated, 6.9). When taken at 15 days after breeding, the mean fetal weight was also decreased in irradiated litters (controls, 0.81; irradiated, 0.63) and so was the crown–rump measurement. The incidence of "anomalies" was 56% in the irradiated group and 37% in the control group. The severity of these responses has not been replicable at such nonthermal or subthermal power densities by other workers. As in the report by Pobzhitkov *et al.* (Po61), we see here values for control mice very different than seen in the U.S. In our studies using CD-1 virgin female mice, the overall sham values for the mean number alive ranges from 10.1 to 11.2 per litter; Bereznitskaya reported a mean value of 7.8 for the same variable. Whether due to genetic derivation, husbandry, nutrition, or other factors, it appears that the term "white mice" may denote some very different strain to the strain (CD-1) used conventionally in the U.S. and in our experiments. The results seen in the Soviet data noted here might find nonreplicability in other countries due to even such minor differences.

Reports of studies into the reproductive effects of microwaves began in earnest in the U.S. in 1970. In 4 experiments, Laskey *et al.* (La70) exposed bred rats with 2.45 GHz CW microwaves, 100 mW/cm^2, for 8–13 min once on day 2, day 2 and day 5 (pre-implantation stage), day 8 (organogenesis stage), or day 15 (fetal stage). These thermal levels of exposure produced group mean rectal temperatures ranging from 41.4 to 42.8°C (sham = 38.0°C). No changes occurred in the pre-implanted embryos but an increased number of resorbed fetuses exposed on day 8 (32 resorptions in 23 exposed litters vs 13 resorptions in 53 sham litters) and a decrease in weight of live fetuses exposed on day 8 and day 13. No obvious abnormal morphology was seen in any of these rat litters when examined on day 19 of gestation. Baranski and Czerski refer to such power intensities as "high levels of exposure, causing death from overheating in the majority of laboratory animals in a relatively short time" (Ba76, p. 83). Even at such thermal loading which produced rectal temperatures of approximately 42°C in 8–13 min, an almost fatal heat load, no teratologic fetuses were observed in 59 exposed pregnant rats.

Brent and his co-workers (Br71, Br72) also studied the effects of 2.45 GHz on the rat pregnancy. Using a modified microwave oven, he exposed one exteriorized uterine horn in each of over 400 pregnant and anesthetized dams to unspecified power densities sufficient to increase the temperature of that horn to 40–49°C in 1–30 min. Unfortunately, no data was published in the available abstracts, except that there were several time-temperature combinations which resulted in 100% resorptions when these fetuses, irradiated on day 9 of pregnancy, reached term. Interestingly, the authors propose the use of this technique in the study of fetal metabolic, physiologic, and toxicologic responses at above normal temperatures.

At the international symposium, "Biologic Effects and Health Hazards of Microwave Radiation" in 1973 in Warsaw, Gordon (Go74) summarized the results of U.S.S.R. research with mice exposed chronically (2.5–48 weeks) to 10 mW/cm^2 in its effort to examine the effects on fetal development. The changes observed were tersely characterized as "increased intrauterine lethality and frequent anomalies in fetal development". At this same meeting, Rugh (Ru74) presented the results of his studies of CF_1 mice exposed to 2.45 GHz CW on day 8 of pregnancy. The forward power was 123 mW/cm^2 delivering "moderately to severely stressful" doses to these pregnant mice for 2–5 min, with a range of 2.5–8 cal/g. The authors concluded that microwave radiation can be teratogenic; that the effects include exencephaly (a teratologic classification very similar to cranioschisis) and decreased fetal weight; that the phenomenon is not all-or-none; that there is a dose–effect relationship; and that there does not appear to be a threshold. More conservative conclusions were reached by these authors in a second paper bearing the same results (Ru75), especially as regards the threshold and dose–effect relationship statements.

Dietzel also demonstrated the effects of loading the fetus thermally with non-ionizing radiation (Di72, Di75). Using a diathermy unit, he exposed a large number of pregnant rats to sufficient power densities (27.12 MHz; 55, 70, or 100 W) to raise their rectal temperatures to 39, 40, or 42°C. The rats were exposed once from day 1 to 16 and he examined the fetuses near term. He found that the peak of sensitivity, as regards the incidence of anomalies, to be days 13–14, when 16% of the fetuses were morphologically abnormal. In this age group, a rise of the dam's temperature to a high of 40°C caused a malformation rate (tail and extremities only) of 12.4%; a dam's rectal temperature of up to 42°C was associated with a tail and extremity malformation rate of 46%. Severe losses occurred before implantation (over 50% in those dams irradiated on day 1 plus day 2) and in late pregnancy (over 20% in dams irradiated on day 15 plus day 16). This fetal wastage, like in the studies by Rugh above, is clearly associated with a general body temperature increase in the dam.

Employing a multimodal cavity at 2.45 GHz, Chernovetz *et al.* (Ch75) irradiated pregnant mice with a single "intense" dose of 38 mW/cm^2 for 10 min (22.8 J/g) on days 11, 12, 13, or 14. The exposure during late

organogenesis or early fetal stage caused no change in fetal mortality or morbidity when compared to shams. Considering the small sample used in each cell of the experimental design (5 pregnant mice/day of pregnancy/sham or irradiated group), the authors' expectation for a statistically valid difference should have been null, even though the dams' mean temperature reached almost 41°C in 10 min of exposure. In another study of the pregnant rat, Chernovetz *et al.* (Ch77) noted an increased rate of resorptions after exposure once on days 10–16. The conditions included multimodal cavity (2.45 GHz) irradiation at 30 mW/cm^2 for 20 min, approx twice the dose applied in the preceding experiment with mice. They concluded that microwaves, as a thermal stressor, cause different effects than more conventional (infrared radiation) sources.

In studies of effects of *in utero* exposure to pregnant rats, Michaelson *et al.* (Mi76) found no adverse effects on the dam or offspring when gestation length or litter size variables were examined. These negative effects were seen in an unspecified number of Long–Evans rats exposed to 2.45 GHz CW at 10 or 40 mW/cm^2 for 1 hr on day 9 (organogenesis) or day 16 (fetal stage) of gestation as part of a larger study of postnatal maturational indices.

Continuing in the examination of the teratologic nature of microwaves in mice, Rugh and McManaway (Ru76a) concluded that day 8 of gestation is the most sensitive to microwave radiation, as it is to ionizing radiation. Using the same waveguide apparatus as in his earlier reports (Ru74, Ru75) at mean absorbed dose rates of 99–115 mW/g, they exposed at least 10 pregnant mice once on each of days 0–11. Because of the high average absorbed dose (24–27.5 J/g in 4 min), they observed 3 times as many fetal deaths and anomalies in day 8 exposed fetuses as in controls, including 10 times (10%) the number of exencephaly. In a further study (Ru76b), these researchers demonstrated that lowering the dam's body temperature with nembutal anesthesia could prevent the teratogenic effects of thermal loading with microwaves. In this important examination of the consequences of over-heating, they used similar exposure conditions as previously reported.

Jensch *et al.* (Je77) used an exposure of 10 mW/cm^2 at 915 MHz on days 1–14, 8 hr daily, on ten pregnant rats. There was no rectal temperature increase caused by the irradiation, nor were there alterations in those variables usually examined for teratologic effects.

In examing the results of our study against the background of available literature on the teratologic effects of microwaves, one must keep in mind the conditions of each experiment, how these have influenced the outcome, and how one microwave study might be incomparable to any other. We have seen how the efforts by Rugh (Ru76a) have demonstrated a peak of sensitivity in the mouse fetus to microwaves (day 8) and that this sensitivity is expressed partly in the increase of exencephaly. In rats, Dietzel (Di72, Di75) found that the most sensitive time is during later gestation and that the tail and extremities are most often affected. Both these investigators used sub-lethal power densities, though with very different frequencies. The different results are due possibly to a species difference. If teratogenic levels are reached in a fetus, its teratologic response will depend upon the stage of development when irradiated. Exencephaly in the mouse would not be caused by treatment after day 12. Tail and extremity anomalies can be caused by earlier application of teratologic agents, but in later pregnancy not many other organogenesis mechanisms are still available to demonstrate anomalous morphology.

The variable response by species may not only be due to the difference in developmental chronology. At the same frequency, i.e. 2.45 GHz, the rat and mouse will have very different absorption rates at any given power density, though this has not yet been demonstrated experimentally using teratologic variables. Also, the rat has a low incidence of spontaneous malformation; major malformation in the CD-1 mouse is 0.84% and in the CD rat only 0.41% (Pa72). Existing, as well, are the differences in strains or

the genetic composition of any experimental group. This is seen in the difference noted above in the fertility of the CD-1 mouse and the mice used by the Soviets in their microwave studies. As regards the capability to sustain thermal stress, the mouse is less stable in its body temperature than the rat. Being that mice are less homeothermic might well influence the outcome of experiments with mice. Also of importance is the sample size under test. A number of the reports noted above suffer in their contribution because of sample sizes too low to have any but the most extreme insult demonstrable as teratogenic. For instance, even if the incidence of major anomalies is tripled due to the application of microwaves to pregnant CD rats, only 1.2% of the fetuses might be expected to exhibit these anomalies. With a litter size of approximately 10, a treated sample population of dams would need to be in excess of 10 to even expect one such anomaly to be seen.

Within logistic confines, we have attempted in this study to remove many of the factors which may have caused confusion in the examination of microwaves as a teratogen. Our use of the outbred CD-1 mouse as the experimental subject conforms to the needs described above for a conventional strain. This particular strain is conveniently available in both the U.S. and Europe. The mice were bred in our laboratory, using virgin females, to ensure the least stress on the pregnancy. The contribution of any male was limited, ruling out a bias due to selection of male fertility. As our prime interest was to examine the effects of microwaves on organogenesis, no particularly susceptibile day of that important stage was selected. The exposure was as chronic as logistically possible considering that the animals needed to be deprived of feed and water during exposure. The sample size was as large as feasible, and was a most important contribution to the validity of the results. Dose rates were kept to the levels where no obvious thermal stress existed in the dam.

The data in this series of experiments appear to indicate that the mouse fetus is responsive to levels of 2.45 GHz CW microwaves that do not cause, at the same time, a significant increase in its dam's rectal temperature. The mean rectal temperatures ranged, before exposure, from 37.9 to 38.7°C (Table 6). These values represent the rectal temperatures of mice which have been awakened from sleep in their maintenance cage and are now active and exploring their new surroundings, i.e. the plastic cage used here to contain each animal individually. By the time the 100-min treatment period had ended, their exploration activity had stopped and their mean temperatures, except for those exposed to 28 mW/cm^2, had decreased 0.4–0.9°C. The exceptional group's mean temperature had increased an insignificant 0.1°C. It does not appear that this post-exposure temperature has any physiological significance as hyperthermia because it is well within the range of mean pre-exposure values.

The anomalies seen in this study are interesting in a number of aspects (Table 5). Most important, perhaps, is the occurrence of cranioschisis in the irradiated fetuses only. The combined rate was approximately 7/3400 live irradiated fetuses and 7/3900 total irradiated implants (extrapolated to 21/10,000 live and 18/10,000 total implants). In the sham group the combined rate for cranioschisis was zero in even larger samples. The high rate of occurrence of cranioschisis seen in the irradiated fetuses and the consistency in which cranioschisis appeared in irradiated fetuses only is strongly suggestive that the application of microwaves was the cause.

No other anomaly but cranioschisis could be shown to be statistically significant, but the occurrence of summed anomalous litters in the irradiated groups totaled 27 while only 12 litters occurred in sham groups (Table 5). This incidence is statistically significant ($P = 0.008$). The greatest contribution to this uneven distribution is from the fetuses in the 3.4 mW/cm^2 power density experiment. There the unusually high incidences of hematoma and club-foot, especially in the irradiated litters, yielded a statistically significant ($P = 0.009$) difference. We feel that this uneven distribution is spurious, especially when no such occurrence is seen at 4 times this power density. Also, no other effect, i.e. stunting,

was seen at 3.4 mW/cm^2 to validate the uneven data distribution. At this stage in our experiments with the teratologic nature of microwaves, we are not fully prepared to accept as due to microwaves only the production of a broad panorama of anomalies.

The litter mean live fetal weight mean value for shams in the 28 mW/cm^2 power density was 0.97 g (Table 3). The irradiates in this group had a mean value of 0.89 g, and these two were significantly different (P = 0.0008). Also, the range of all other means of this variable was 0.96–1.01 g. It is strongly suggestive, then, that the low value is the result of exposure to 28 mW/cm^2.

SUMMARY

The significant effects seen in mouse fetuses as a result of exposure to 2.45 GHz CW for 100 min daily at a power density range of 3.4–28 mW/cm^2 were: (1) the appearance of cranioschisis, and (2) stunting at the highest power density. This was achieved at a specific absorption rate of 1.2–13.3 J/g with no physiologically significant increase of the maternal rectal temperature, and represents no obvious general maternal thermal response. It appears that microwaves at the range used in this study, especially 28 mW/cm^2, are embryopathic.

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